Synopsis

Green synthesis of capsaicin-based nanoparticle from tissue cultured Bhut Jolokia chilli plant to control Anthracnose disease

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STATE OF ART

Chilli is one of the major economically important vegetable crops belongs to the family Solanaceae and cultivated throughout the world. In chilli production, India is the world's largest producer and exporter of dry chillies, with an output of 4.7 million tonnes (FAOSTAT, 2023). Bhut Jolokia chilli plant (*Capsicum chinense* Jacq) is commonly known as the ghost pepper and it is one of the hottest chilli varieties in the world with capsaicin content ranging from 1,000,000 to 1,041,427 Scoville Heat Units (SHU)[1]. Capsaicin, a bioactive compound primarily found in chili peppers of the *Capsicum* genus, has garnered considerable interest due to its wide-ranging pharmacological and agricultural properties. Well-known for its characteristic pungency, this alkaloid demonstrates potent antimicrobial, antifungal, antioxidant, anticancerous and anti-inflammatory effects, positioning it as a promising candidate for diverse applications.[2-4] Bhut Jolokia chilli plant mainly grown in northeast region of India like Assam, Nagaland, Manipur, Mizoram and it is called by several vernacular names such as Bhut Jolokia, Bih Jolokia, Dhan Jolokia, Naga Jolokia, Krishna Jolokia, Lota Bhut, Mem Jolokia. The indigenous communities of Northeast India utilize this plant both as a culinary ingredient and as a traditional remedy for various ailments, including tonsillitis, ulcers, and dysentery[5,6] The concentration of bioactive compounds, including capsaicin, in the Bhut Jolokia plant is significantly influenced by the interaction between its genotype and environmental factors.[7] Leveraging its unique chemical profile, the synthesis of capsaicin-based nanoparticles offers a novel approach for addressing pressing challenges in plant disease management.

Anthracnose is one of the most destructive fungal diseases affecting a range of economically important vegetable crops such as tomato, pea, chilli, brinjal, beans etc.[8,9] The disease is primarily caused by *Colletotrichum* species, such as *C. gloeosporioides*, *C. capsici*, and *C. acutatum*, which thrive in tropical and subtropical climates. Symptoms include dark, sunken necrotic lesions on fruits, stems, and leaves, leading to significant yield losses.[10] Traditional control methods, including chemical fungicides, raise concerns due to environmental toxicity, pathogen resistance, and residue accumulation on crops.[11] Therefore, there is an urgent need for eco-friendly and effective strategies to mitigate this disease.

Nanotechnology, an interdisciplinary field with applications spanning agriculture, medicine, and materials science, has emerged as a transformative tool in plant disease

management. Nanoparticles, owing to their high surface area-to-volume ratio and tunable physicochemical properties, enhance the bioavailability and targeted delivery of active compounds[12]. Capsaicin-based nanoparticles could provide a dual advantage: suppressing fungal pathogens while reducing the environmental footprint of traditional fungicides.[13]Initial studies have demonstrated the potential of capsaicin to inhibit fungal growth by disrupting cell membranes and altering metabolic pathways.[14] However, its direct application faces challenges such as limited stability and poor solubility, which can be addressed through nanoparticle formulation.

Additionally, the multiplication of Bhut Jolokia chilli plant face challenges such as low seed viability and reduced germination rates. Plant Tissue culture allows for the consistent production of high-quality Bhut Jolokia plants, ensuring a sustainable and controlled source of capsaicin.[15,16]Plant tissue culture provides controlled *in vitro* environment for growing a plant and for plants like chilli it can be highly beneficial.

Integrating tissue-culture with nanotechnology, it is possible to develop a scalable and sustainable method for managing various diseases in vegetable crops. Nanotechnology involves the design, production, and application of materials and devices at the nanometer scale.[17,18] It has transformed fields like medicine, agriculture, and environmental science by enabling precise and efficient solutions.[19] In agriculture, nanotechnology offers innovative approaches for crop protection, disease management, and nutrient delivery, ensuring sustainability and reduced environmental impact.[20,21]Studies on nanoparticle-based fungicides, such as silver and zinc oxide nanoparticles, have demonstrated significant antifungal activity against *Colletotrichum* spp.[22] However, the use of natural, plant-derived bioactive compounds, such as capsaicin, in nanoparticle systems for anthracnose control has not yet been extensively documented.

PROBLEM TO BE INVESTIGATED

The problem to be investigated is the development of a sustainable and eco-friendly approach to control Anthracnose disease, a severe fungal infection caused by *Colletotrichum* species that significantly reduces the yield and quality of several plants. Current chemical-based fungicides, while effective, pose serious environmental and health risks due to their toxicity, persistence in the ecosystem, and potential to induce pathogen resistance. Moreover, the high demand for organic and safe agricultural produce necessitates the exploration of greener alternatives. Tissue

cultured Bhut Jolokia plants offer a uniform and disease free platform for experimentation, ensuring consistency and reproducibility of results. Capsaicin, the active compound in chilli peppers, is known for its antimicrobial and antifungal properties, making it a promising candidate for developing biocontrol strategies. However, its direct application is limited by issues such as low bioavailability, stability, and targeted delivery. Nanotechnology presents an innovative solution by enabling the synthesis of capsaicin-based nanoparticles, which can enhance the compound's efficacy through improved stability, controlled release, and targeted action against fungal pathogens. The investigation focuses on utilizing green synthesis methods to produce these nanoparticles, leveraging plant-based precursors from tissue-cultured Bhut Jolokia for an environmentally sustainable process. This approach aligns with the global push toward minimizing chemical inputs in agriculture while addressing the urgent need for effective, eco-friendly solutions to manage Anthracnose disease, thus contributing to enhanced productivity, reduced crop losses, and sustainable farming practices.

OBJECTIVE OF THE INVESTIGATION & MOTIVATION

Objectives of this investigation

1. To establish a tissue culture protocol for Bhut Jolokia plant.
2. Extraction of capsaicin from tissue-cultured Bhut Jolokia plants.
3. Green synthesis and characterization of capsaicin-based nanoparticles.
4. To evaluate the efficacy of the formulated nanoparticles against Anthracnose disease under *in vitro* condition.

Motivation behind this research

The motivation behind this research stems from the critical need to address the challenges posed by Anthracnose disease in vegetable crops, which significantly impacts the yield and quality of these crops. This disease, caused by *Colletotrichum* species, leads to substantial economic losses for farmers and threatens the sustainability of yield production. Existing chemical fungicides, while effective, raise serious concerns due to their environmental toxicity, health risks, and the development of resistant fungal strains, underscoring the urgent need for safer and eco-friendly alternatives. Bhut Jolokia, renowned for its extreme pungency and high capsaicin content, holds significant cultural, economic, and medicinal importance. Its unique properties make it an ideal candidate for exploring innovative, natural disease-control strategies. The antimicrobial and antifungal potential of capsaicin presents an opportunity to develop plant-based solutions;

however, its direct use faces challenges such as poor stability, bioavailability, and lack of targeted action. Nanotechnology provides a promising avenue to overcome these limitations by enhancing capsaicin's efficacy through nanoparticle synthesis. Green synthesis, particularly using plant-based precursors like tissue-cultured Bhut Jolokia, offers a sustainable and environmentally friendly method that aligns with global efforts to promote organic and sustainable agricultural practices. By developing capsaicin-based nanoparticles, this research aims to provide an effective, eco-friendly solution to combat Anthracnose disease, reduce reliance on harmful chemicals, and support sustainable chilli production. Additionally, the study contributes to advancing green nanotechnology and demonstrates the potential of tissue-cultured plants as a renewable resource for novel agricultural innovations.

HYPOTHESIS

In the current study, we hypothesized that capsaicin-based nanoparticles synthesized through a green approach using tissue-cultured Bhut Jolokia plants will exhibit enhanced antifungal activity against *Colletotrichum* species, the causative agent of Anthracnose disease, by improving the stability, bioavailability, and targeted delivery of capsaicin, thereby providing an eco-friendly and effective alternative to conventional chemical fungicides in cultivation of vegetable crops.

RESEARCH DESIGN AND METHODOLOGY

A. Materials

1. **Plant Material**

   Bhut Jolokia seeds (*Capsicum chinense* Jacq) will be obtained from a certified seed center or seed bank.
2. **General Chemicals:** MS media, Phytohormones, Ethanol, PDA, Acetonitrile, Distilled water, Methanol etc.
3. **Tools to be used:**

   Glassware: Measuring cylinder, Culture tube, Pipette, Petri plates etc.

   Equipment and machines: pH meter, Autoclave, Weighing machine, Spatula, Laminar Air Flow, Forceps, Burner, B.O.D Incubator, spectrophotometer, XRD, SEM, HPLC etc. and other tools as per the need of the work.

B. Methodology

1. **Tissue Culture of Bhut Jolokia:** For the *In vitro* multiplication, we follow Mahto et al. (2018)[16] protocol with slight modification and develop a highly efficient regeneration protocol for Bhut Jolokia chilli plant by using leaf, internode and axillary bud as an explant. we have to optimize with the various type of phytohormones combinations and concentration particularly of cytokinins and auxins in plant growth medium, and the culture conditions light and temperature.

2. Extraction and quantification of Capsaicin: Capsaicin extraction will follow the method of Collins et al. (1995)[23]with slight modifications. Two hundred milligrams of ground tissue sample will be extracted using 10 mL of pure acetonitrile. The suspension will be incubated at 80°C in a water bath for four hours. After incubation, the mixture will be cooled to room temperature and filtered through a PVDF membrane filter (Millipore). The filtered extract will be stored at 4°C until use. To prevent degradation, the samples will be analyzed within 2–3 days of preparation. Extracted capsaicin will quantify by using HPLC.

3. Synthesis of Capsaicin based nanoparticle and characterization: Capsaicin-based nanoparticles will be synthesized using the nanoprecipitation method, where purified capsaicin extract will be dissolved in an organic solvent such as ethanol, and a biodegradable polymer, such as chitosan or poly lactic-co-glycolic acid (PLGA), will be dissolved in an aqueous solution under constant stirring. The organic phase containing capsaicin will then be added dropwise into the aqueous phase, resulting in the self-assembly of nanoparticles due to solvent diffusion and polymer precipitation. The mixture will be stirred continuously for stabilization, and the solvent will be evaporated under reduced pressure. The synthesized nanoparticles will be characterized for particle size and morphology using dynamic light scattering (DLS) and scanning electron microscopy (SEM), while encapsulation efficiency and capsaicin loading will be determined using high-performance liquid chromatography (HPLC). Surface charge and stability will be assessed through zeta potential analysis, and functional group interactions will be examined using Fourier-transform infrared spectroscopy (FTIR).

4. *In vitro* efficacy and antifungal activity: The *in vitro* efficacy and antifungal activity of capsaicin-based nanoparticles will be evaluated using healthy Capsicum plants grown under controlled conditions. Plants will be artificially inoculated with a spore suspension of *Colletotrichum* spp., the causative agent of anthracnose disease, by spraying the pathogen suspension on leaves until runoff. After inoculation, capsaicin-based nanoparticles at various concentrations will be applied as foliar sprays, while untreated and pathogen-only plants will serve as negative and positive controls, respectively. Disease progression will be monitored by

measuring lesion size, percent disease index (PDI), and plant health parameters, such as chlorophyll content and biomass, over time. The antifungal activity of nanoparticles will also be assessed *in vitro*, where fungal mycelial growth will be monitored on media containing different concentrations of nanoparticles. The minimum inhibitory concentration (MIC) of nanoparticles will be determined by observing the lowest concentration at which fungal growth is completely inhibited. Statistical analysis will be performed to evaluate the effectiveness of the treatment compared to controls.

5. Data Analysis: During the investigation all data will be analyzed by using various statistical tools.

SIGNIFICANCE OF THE STUDY

The significance of this study lies in its potential to provide an eco-friendly and sustainable solution to manage Anthracnose disease in vegetable crops, which is a major threat to agricultural productivity and farmer livelihoods. By leveraging the unique properties of capsaicin from tissue-cultured Bhut Jolokia plants and advancing its application through green-synthesized nanoparticles, the research addresses critical challenges associated with conventional chemical fungicides, including environmental toxicity, human health risks, and the development of resistant fungal strains. Furthermore, the use of tissue-cultured plants ensures a reliable and consistent source of capsaicin, promoting reproducibility and scalability of the nanoparticle synthesis process. This study contributes to the growing field of green nanotechnology by integrating plant-based resources into nanoparticle synthesis, aligning with global efforts to minimize chemical inputs in agriculture. The findings have the potential to benefit by reducing crop losses and production costs but also consumers by supporting the production of safer, organic agricultural products. Additionally, the research serves as a model for exploring plant-derived bioactive compounds and nanotechnology for disease management in other crops, fostering sustainable agricultural practices and environmental conservation.

IT'S RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED OF THE SOCIETY

This study is highly relevant to addressing the growing need for sustainable and eco-friendly solutions to combat Anthracnose disease, a major threat to global agricultural productivity. The overuse of chemical fungicides has resulted in environmental degradation, health risks, and

resistant fungal strains, underscoring the urgency of finding safer alternatives. By utilizing tissue-cultured Bhut Jolokia plants for the green synthesis of capsaicin-based nanoparticles, this research offers a natural and effective approach to disease management. Capsaicin's antifungal properties, enhanced through nanoparticle technology, provide improved stability, bioavailability, and targeted action against *Colletotrichum* species, reducing reliance on synthetic fungicides. This aligns with the societal demand for organic and sustainable agricultural practices, benefiting farmers by minimizing crop losses and consumers by promoting safer food production. Additionally, the study demonstrates an innovative, scalable approach to integrating plant-based resources and nanotechnology for sustainable crop protection solutions.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

This study contributes to existing knowledge by advancing the application of green nanotechnology in agricultural disease management, specifically for controlling Anthracnose disease in vegetable crops. It introduces a novel approach to synthesizing capsaicin-based nanoparticles using tissue-cultured Bhut Jolokia plants, enhancing the bioactivity and stability of capsaicin for improved antifungal efficacy. The research expands the understanding of how plant-derived bioactive compounds, when integrated with nanotechnology, can offer an environmentally sustainable alternative to conventional chemical fungicides. Additionally, the study provides insights into the potential of plant tissue culture as a reliable, renewable resource for the green synthesis of nanoparticles, promoting sustainability in both plant biotechnology and nanotechnology. By demonstrating the effectiveness of these nanoparticles against *Colletotrichum* species, the research contributes to the development of targeted, eco-friendly solutions in crop protection, enriching the broader field of plant-based nanomaterials and their applications in agricultural practices.

FUTURE PERSPECTIVE

The future perspective of this research lies in further exploring the scalability and commercial viability of capsaicin-based nanoparticles synthesized from tissue-cultured Bhut Jolokia plants, with the potential to expand their application for the management of various fungal diseases in different crops, thereby contributing to the development of more widespread, sustainable agricultural practices. Additionally, future studies could focus on optimizing the synthesis process, improving nanoparticle stability, and evaluating the long-term environmental and ecological impact of these bio-based nanomaterials, as well as investigating their synergistic

effects when combined with other natural or biological agents for enhanced disease control. Expanding the understanding of capsaicin's mechanisms of action at the molecular level and assessing its efficacy in real-world agricultural settings will provide valuable insights for integrating such nanotechnological innovations into integrated pest management strategies, promoting a shift toward more eco-friendly, organic farming practices.

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